

Section A7.1.1.2.1 Biodegradability (Closed Bottle Test, OECD 301 D)
Section A7.1.1.2.2

Annex Point IIA7.6.1.1

		1 REFERENCE
1.1	Reference	van Ginkel, C.G. and C.A. Stroo, 2000, Biodegradability of Preventol A4-S in the Closed Bottle Test, Akzo Nobel, Chemicals Research, Arnhem, NL, Report No. CGS-ENV F00057 T 00003 C, 2000-02-16.
1.2	Data protection	Yes
1.2.1	Data owner	Bayer Chemicals AG
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	Guideline study	Yes, Closed Bottle Test = OECD 301D (=EC Test Guideline C.6)
2.2	GLP	Yes
2.3	Deviations	Yes, the test was prolonged because the pass level was not reached at day 28.
		3 MATERIALS AND METHODS
3.1	Test material	Dichlofluanid (Preventol A4-S) = N-(dichlorofluoromethylthio)-N',N'-dimethyl-N-phenylsulfamide
3.1.1	Lot/Batch number	██████████
3.1.2	Specification	As given in Section 2 of the dossier
3.1.3	Purity	██████
3.1.4	Further relevant properties	low water solubility: 1.3 mg/l
3.1.5	Composition of Product	-
3.1.6	TS inhibitory to micro-organisms	EC ₅₀ = 19 mg/l (respiration inhibition in activated sludge)
3.1.7	Specific chemical analysis	The test substance was characterised by HPLC, external standard
3.2	Reference substance	Yes, Sodium benzoate
3.2.1	Initial concentration of reference substance	2 mg/l
3.3	Testing procedure	
3.3.1	Inoculum / test species	Secondary activated sludge (2 mg dry weight/l) from the WWTP Nieuwgraaf in Duiven, Netherlands
3.3.2	Test system	Test was performed in 250 to 300 ml BOD (biological oxygen demand) bottles. The dissolved oxygen concentrations were determined electrochemically using an oxygen electrode and meter.

Official
use only

X

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		pH and temperature were also measured.	
3.3.3	Test conditions	Bottle 1-10: activated sludge + silica gel Bottle 11-20: Dichlofluanid + activated sludge + silica gel Bottle 21-26: Sodium benzoate + activated sludge Bottle 27-32: only activated sludge pH of the media: 7.0 at the start of the test; 6.8 at day 28 Temperature: 21 ± 1 °C	
3.3.4	Method of preparation of test solution	The test substance was first dissolved in dichloromethane (1 g/l). The test substance in dichloromethane (0.60 ml) was added to silica gel in petri dish. The solvent was allowed to evaporate and the entire contents were transferred to the BOD bottle. The nominal concentrations were not analytically confirmed.	X
3.3.5	Initial TS concentration	2 mg/l dichlofluanid	
3.3.6	Duration of test	28 days; prolonged to day 140 by measuring the course of the oxygen decrease in the bottles of day 28 using a special funnel	
3.3.7	Analytical parameter	The amount of oxygen taken up by the microbial population during biodegradation of the test substance is expressed as BOD (biological oxygen demand) and ThOD (Theoretical oxygen demand).	
3.3.8	Sampling	Day 7, 14, 21, 28, 42, 56, 84, 112 and 140	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	Not performed, not relevant due to the lack of degradability	
3.3.11	Controls	Control without test substance; abiotic controls	
3.3.12	Statistics	n.a.	

4 RESULTS

4.1	Degradation of test substance		
4.1.1	Graph	Provided in the report	
4.1.2	Degradation	9% degradability after 28 days. Since, in the prolonged test dichlofluanid is biodegraded 41% at day 140, it should be classified as inherently biodegradable.	X
4.1.3	Other observations	Inhibition of the endogenous respiration of the inoculum by dichlofluanid was not detected. Therefore, no inhibition of the biodegradation due to the "high" initial concentration of dichlofluanid is expected.	
4.1.4	Degradation of TS in abiotic control	No abiotic control with dichlofluanid	
4.1.5	Degradation of reference substance	53% degradability after day 7, 71% degradability after day 14	
4.1.6	Intermediates/ degradation products	n.a.	

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		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	The test was performed according to the OECD test guideline No. 301D (Closed Bottle Test).	
5.2	Results and discussion	Dichlofluanid was biodegraded 9% at day 28 in the Closed Bottle Test. In the prolonged Closed Bottle Test the compound was biodegraded 41% at day 140. The biodegradation reached at day 140 of the test demonstrates that dichlofluanid should be classified as inherently biodegradable. The test compound is probably partially degraded in the prolonged test. The reference substance, sodium benzoate, was degraded 71% after 14 days.	X
5.3	Conclusion	Validity criteria can be considered as fulfilled: The endogenous respiration was 1.5 mg/l; the oxygen concentrations > 0.5 mg/l in all bottles during the test period.	
5.3.1	Reliability	Reliability indicator = 1	
5.3.2	Deficiencies	No	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25/11/2004
Materials and Methods	<p>Applicant's version is acceptable with the following comments:</p> <p>3.3.1 The inoculum / test species was secondary activated sludge, aerated to precondition it and reduce endogenous respiration rates.</p> <p>3.3.4 As the method of preparation of the test solution was non-standard information on the stability of dichlofluanid during evaporation, and its rate of release from the silica (which could affect its rate of biodegradation) should have been reported. The initial test concentrations were not recorded. The temperature and pH were monitored, with the latter being around pH 6.9 rather than pH 7.4 as expected, but ammonium had been left out of the medium to prevent nitrification.</p>
Results and discussion	<p>Applicant's version is acceptable with the following comment:</p> <p>4.1.2 Preventol A4-S showed 9% degradation after 28 days, but no degradation at 42 days, and hence appears to be correctly assigned as 'not readily biodegradable'. Following continuation of the test to 140 days further biodegradation of Preventol A4-S was shown after about 50 days, and therefore this substance may be inherently biodegradable.</p>
Conclusion	<p>Applicant's version is acceptable with the following comment:</p> <p>5.2 The method reported is considered adequate to demonstrate that dichlofluanid is not classified as 'readily biodegradable', but that there was evidence that the substance underwent inherent primary degradation.</p>
Reliability	1
Acceptability	Acceptable
Remarks	All endpoints and data presented in the summary and tables have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	